DEVELOPMENT OF MULTIPLE INTERACTIONS MIXED MATRIX MEMBRANE CHROMATOGRAPHY USING LEWATIT MP500 ANION RESIN AND LEWATIT CNP 105 CATION RESIN FOR WHEY PROTEIN FRACTIONATION

CHAN FONG SHIEW

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α-Lac	α-lactalbumin
β-Lac	β-lactoglobulin
AC	Affinity chromatography
BOD	Biochemical Oxygen Demand
BSA	Bovine serum albumin
CA	Cellulose acetate
COD	Chemical Oxygen Demand
Cys	Cysteine
Da	Dalton
DMSO	Dimethylesulfoxide
EVAL	Ethylene vinyl alcohol
GF	Gel filtration
Hb	Bovine hemoglobulin
HIC	Hydrophobic interaction
HPLC	High Performance Liquid Chromatography
IEC	Ion exchange
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LDS	Lithium dodecyl sulfate
LF	Lactoferrin
LP	Lactoperoxidase
MF	Microfiltration
mM	milimolar
MMM	Mixed matrix membrane
NaOH	Sodium hydroxide
NF	Nanofiltration
NMP	N-Methyl-2-pyrrolidone
PEG	Polyethylene glycol
pI	Isoelectric point

RO	Reverse osmosis
RPC	Reversed phase
RPM	Revolutions per minute
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
UF	Ultrafiltration
WPI	Whey protein isolate
Wt/vol	Weight/volume

LIST OF SYMBOLS

€	Euro
µ	Micro
Å	Ångström
α	Alpha
β	Beta

PENGHASILAN MEMBRAN KROMATOGRAFI CAMPURAN MATRIK PELBAGAI INTERAKSI MENGGUNAKAN RESIN ANION LEWATIT MP500 DAN RESIN KATION LEWATIT CNP105 UNTUK PENGASINGAN PROTEIN DARIPADA *WHEY*

ABSTRAK

Cara konvensional untuk pengasingan protein dilakukan dengan mengunakan kromatografi turus terpadat. Namun demikian, teknik ini mempunyai beberapa kelemahan seperti kejatuhan tekanan yang tinggi dan kadar aliran operasi yang terhad. Membran kromatografi dapat mengatasi masalah dalam kromatografi turus terpadat tetapi proses penyediaan membran kromatografi ini memerlukan pengubahsuaiankimia yang melampau. Konsep penyediaan campuran membran matrik (MMM) adalah kaedah alternatif kepada penyediaan membran kromatografi iaitu menggunakan kaedah fizikal dengan mencampurkan resin boleh jerap dengan larutan polimer membran. Di dalam kajian ini, MMM pelbagai interaksi telah dibangunkan untuk pengasingan protein daripada *whey* meggunakan 7.5 wt% CNP105 kation resin dan 42.5% wt% MP500 anion resin relatif kepada kandungan polimer asas membran. Berdasarkan analisa HPLC dan SDS-PAGE, kedua-dua protein *whey* bersifat asid dan alkali telah terjerap kepada MMM pelbagai interaksi dalam satu ujian pengasingan protein daripada *whey*. Kadar penjerapan untuk *whey* protein bersifat asid menggunakan MMM berasaskan EVAL adalah 4.255 mg BSA/ g MMM, 60.887 mg α-Lac/ g MMM dan 231.788 mg β-Lac/ g MMM. Bagi MMM berasaskan CA, kadar penjerapan adalah 2.970 mg BSA/ g MMM, 42.392 mg α-Lac/ g MMM dan 179.817 mg β-Lac/ g MMM.

DEVELOPMENT OF MULTIPLE INTERACTIONS MIXED MATRIX MEMBRANE CHROMATOGRAPHY USING LEWATIT MP500 ANION RESIN AND LEWATIT CNP 105 CATION RESIN FOR WHEY PROTEIN FRACTIONATION

ABSTRACT

The conventional method to purify protein is by using packed bed column chromatography. However, this method had several limitations such as high pressure drop and limited flow rate operation. Membrane chromatography can be used to overcome the limitation of packed column but the preparation of adsorptive membrane requires harsh chemical modifications. Mixed matrix membrane (MMM) preparation concept can be used as an alternative route to prepare membrane chromatography by physical blending of adsorptive resin with membrane polymer solution. In the current research, multiple interactions MMM chromatography was developed for whey protein fractionation using 7.5 wt% CNP105 cation resin and 42.5 wt% MP500 anion resin relative to base polymer content. The resins were blend at different composition in EVAL and cellulose base polymer matrix. Based on HPLC and SDS-PAGE analysis, both acidic and basic whey proteins were bound to the multiple interactions MMM in single run of whey batch fractionation. The binding capacity for major acidic whey proteins using EVAL based MMM are 4.255 mg BSA bound/ g MMM, 60.887 mg α-Lac bound/ g MMM and 231.788 mg β-Lac/ g MMM. For CA based MMM, the binding capacity are 2.970 mg BSA bound/ g MMM, 42.392 mg α-Lac bound/ g MMM and 179.817 mg β-Lac/ g MMM.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Proteins molecule are different in each other in term of size, shape, charge, hydrophobicity and affinity towards other molecules. These properties can be exploit to separate protein mixture into its individual components for commercial use in industry. Generally, proteins separation can be done using various techniques such as centrifugation, electrophoresis, membrane filtration and chromatography based separation.

Chromatography is the most favorable used technique for high resolution proteins separation and purification (Ghosh, 2002). Three common types of interactions in chromatography are ion-exchange chromatography, gel filtration chromatography and affinity chromatography. Among them, ion exchange chromatography is the most widely used techniques in protein downstream processing.

Nowadays, different types of ion exchange resins are commercially available in protein capturing, purifying and polishing steps (Zhou et al., 2002). Ion exchanger chromatography is crucial for recovery and purification of proteins, polypeptides, nucleic acid and other biomolecules. It functions based on the concept of reversible electrostatic interaction between a charged molecule and the oppositely charged chromatographic media (Bhattacharjee et al., 2006). Furthermore, it has an advantage as the elution often takes place at a relatively mild condition so that proteins are able to maintain their confirmation during chromatographic processing (Saiful & Wessling, 2006).

Purification of proteins by chromatography conventionally done by using packed bed chromatography configuration. However, there are several limitations of packed bed chromatography. There is an increasing interest in developing membrane chromatography that offer advantages compared to packed bed chromatography such as high flow rate without loss of adsorption capacity, large scale operation, absence of long diffusion path length and low pressure drops. Membrane chromatography is a membrane that acts as a short and wide chromatographic column with a variety of adsorptive mechanisms such as hydrophobic, ion exchange and affinity interactions (Klein, 2000; Zou et al., 2001).

Mixed matrix membrane (MMM) concept offer simple procedure to prepare membrane chromatography instead of using chemical modification process. It is prepared by incorporating an adsorptive resin into a membrane polymer solution prior to membrane casting (Saufi & Fee, 2011). According to Avramescu et al. (2003a), this concept is simple and flexible whereby the geometry, adsorption

capacity and the functionality of the membranes are easily adjusted. Avramescu et al. (2003b), had prepared cationic MMM by incorporating Lewatit CNP80WS cation resin into EVAL polymer in the form of flat sheet, solid fiber and hollow fiber membranes to study the fractionation of bovine serum albumin (BSA) – bovine hemoglobulin (Hb). Later, this concept has been expanded by Saufi and Fee (2011) to prepare multiple interaction membrane chromatography by using SP-Sepharose cation resin and MP500 anion resin in single membrane material. They used this mixed mode MMM for whey protein fractionation. In this study, another alternative cation resin Lewatit CNP105 will be used to prepare mixed mode MMM to replace the SP Sepharose which is very costly cation resin.

1.2 Problem Statement

Traditionally, packed bed column chromatography is used in protein separation. However, there is limitation found in packed bed column chromatography especially related to very high pressure drop occurs across the column. Besides that, the packed bed column is not able to be operating at high flow rate. This problem can be resolve using membrane chromatography. However, the common method to prepare adsorptive membranes requires a complex process and sometimes modifications of membranes using harsh chemicals is required. Thus, the concept of MMM preparation technique has a potential in preparing membrane chromatography material. Besides that, the current ion exchange membrane chromatography normally can be operated with single interaction either as anion or cation exchanger. Hence, the development of multiple interactions in MMM chromatography can offer advantages in binding both acidic and basic protein

simultaneously from single run. Meanwhile, from the past literature works shown that the cost of SP Sepharose cationic resin is high compared to the proposed cation resin, Lewatit CNP105 used in this study. Thus, it is favorable to replace the expensive cation resins to a more affordable, low cost and same efficiency and performance as the SP Sepharose resin.

1.3 Research Objective

The objective of this research is to develop multiple interactions MMM chromatography for whey protein fractionation using Lewatit CNP105 cation resin and Lewatit MP500 anion resin.

1.4 Scopes of Study

In order to fulfill the objectives of this research, the following scopes have been outlined:

i. Development and characterization of multiple interactions MMM chromatography using Lewatit MP500 anionic resin and Lewatit CNP105 cationic resin with EVAL based matrix.
ii. Feasibility study on the development of multiple interactions MMM chromatography using cellulose-based matrix.

CHAPTER 2

LITERATURE REVIEW

2.1 Whey

In dairy industry, whey is a byproduct of cheese-making and casein manufacture. The remaining watery and thin liquids is called whey after the casein curd separate from the milk and undergo coagulation through the action of enzyme or pH adjustment. The whey is yellowish or greenish in color depending on the type and quality of milk (Smithers, 2008). Mostly, whey can be made from a wide range of milk, with cow's milk being the most popular choice in the area of western countries.

Nowadays, whey is a nuisance and major problem to the cheese making and casein manufacture industry. In the production of cheese industry, almost 10 kg of milk produces 1-2 kg of cheese while the remaining 8-9 kg consists of liquid whey (Bhattacharjee, 2006). The increasing quantity of milk production leads to a larger volume of cheese, casein or caseinate and other dairy products and thus increasing

the volume of whey production. From Figure 2.1, the amount of whey production is increasingly due to the growth of milk industry (Smithers, 2008).

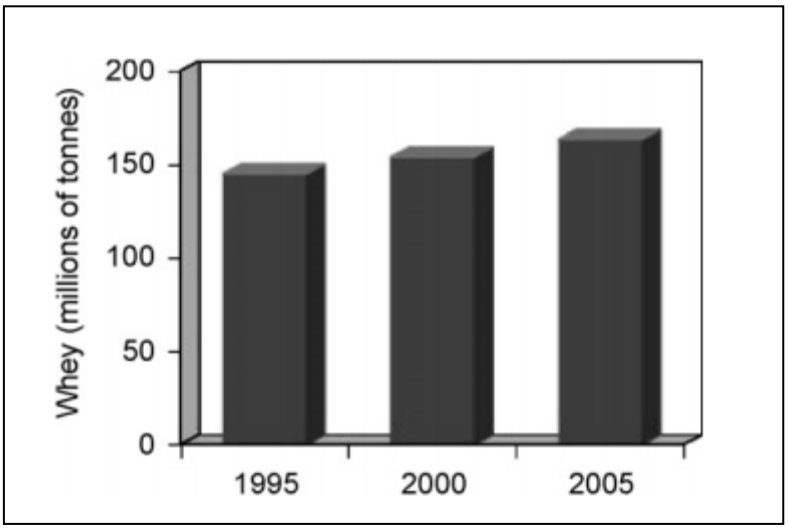

Figure 2.1 Annual volume of dairy whey produced globally (1995-2005). Volume increase over this period shows 1-2% annual growth rate, approximately equivalent to the average annual growth in milk output over this same period (Source: Smithers, 2008)

Whey is a waste product stream and is constantly being disposed and discharged from the industry. There are several disposal method practiced by western country from the previous centuries whereby the cheese-makers and casein manufacturers spray the whey onto fields, discharging through rivers, lakes or ocean, discharging into municipal sewage system or selling it as animal feed (Smithers, 2008). Later, the disposal of whey waste had known to become an issue to environment pollution whereby this waste contaminated the water system. This is proven by an analysis done using biochemical oxygen demand (BOD) and chemical oxygen demand (COD). From the analysis, the BOD value and COD value showed value of 35 000 – 60 000 mg L^{-1} and 80 000 – 100 000 mg L^{-1} each respectively (Bhattacharjee, 2006).

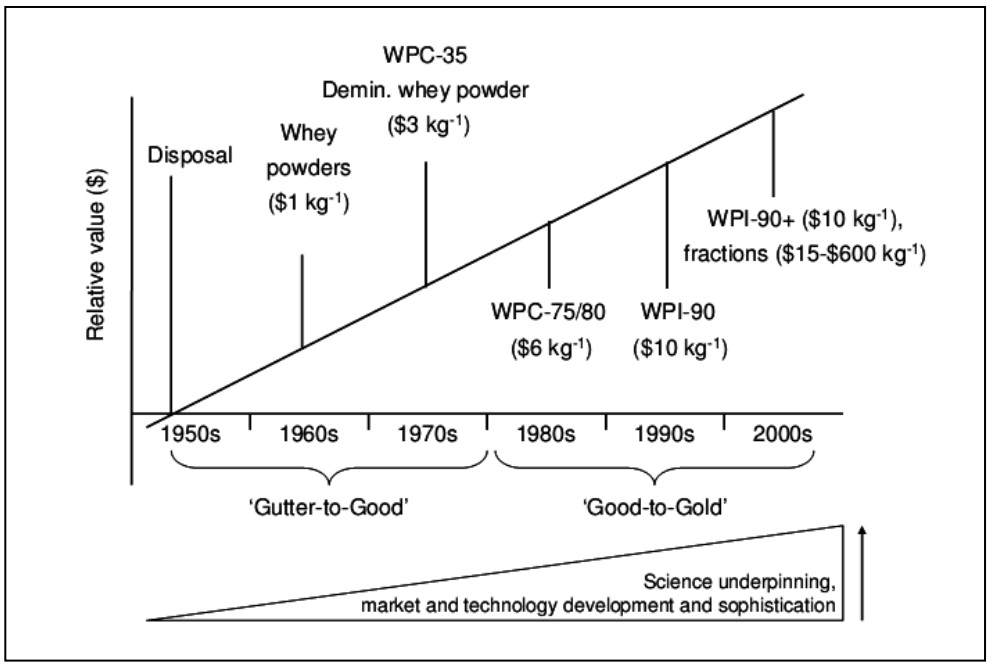

Figure 2.2 Schematic representation of the relative increase in value of whey protein due to advancement of technology and knowledge on the importance of whey protein composition (Source: Smithers, 2008)

In 1950s, whey is considered as waste and is usually being disposed without fully utilization its source of protein. However, with increasing knowledge and advancement of science and technology researchers had found out that whey is an excellent source of proteins that contain all the essential amino acids (Monteiro et al., 2008). Meanwhile, the consumer demand on health product is increasing and thus separation and purification of protein in whey for health supplement is a must. As a consequence, there is an increase in terms of relative price value for whey protein as illustrated from Figure 2.2. The relative value of whey protein for the past 50 years is increasing from $1 per kg at 1960s to $10 per kg at year 2000.

2.2 Whey Protein

The major whey protein components comprises of β-lactoglobulin (β-Lac), α-lactalbumin (α-Lac), bovine serum albumin (BSA), lactoferrin (LF), lactoperoxidase (LP), glycomacropeptide and etc (Bhattacharjee, 2006). Table 2.1 shows the composition and properties of whey proteins.

Table 2.1 Composition of whey protein (Source: Andersson & Mattiason, 2006)

	Concentration (kg m^{-3})	Isoelectric point (pI)	Molecular weight (kDa)
β-Lactoglobulin	2-4	5.2	18
α- Lactalbumin	1.2-1.5	4.5-4.8	14
Bovine serum albumin	0.3-0.6	4.7-4.9	69
Immunoglobulins (IgG, IgA, IgM)	0.6-0.9	5.5-8.3	150-1000
Lactoperoxidase	0.02-0.05	9.5	78-79
Lactoferrin	0.02-0.2	8-9.5	78-92

There are two types of processed whey protein which are whey protein concentrates and whey protein isolates. Whey protein concentrates are rich in whey proteins but contain fat and lactose. This whey protein concentrates are obtained from membrane filtration of whey. Meanwhile, whey protein isolates contain whey protein with low fat and lactose. It is produced through rigorous and complex separations of whey such as a combination of ultrafiltration and microfiltration or ion exchange chromatography (Etzel et al., 2008).

Most of the whey protein components have their own high commercial values in the market and application both in health and food industry. It is known that the world production of cheese whey per year is estimated as 130 million tons. This means that the global production is equivalent to 780 000 tons of proteins which in

turn for separation and protein purification will benefits the economic growth (Monteiro, 2008). Table 2.2 summarizes the importance functions of composition protein found in whey.

Table 2.2 Importance functions of protein compositions in whey protein fractionation

Compositions	Functions	References
β-Lactoglobulin	- Better foam stabilizer used in the production of confectionary due to its food gelling properties - Transport or accumulation of lipid-soluble biological components such as fatty acids and retinols - Rich source of Cys that stimulate glutathione synthesis, an anticarcinorgenic tripeptide produced by the liver for protection against intestinal tunors	Mcintosh et al., 1995; Zydney, 1998; Cowan & Ritchie, 2007; Madureira et al., 2007; Amigo et al., 2008
α-Lactalbumin	- Used in infant formula due to high tryptophan content - Enhanced whippability in meringue like formulations - Potential as contraceptive agents due to strong affinity for glycosylated receptors on the surface of oocytes and spermatozoids - Preventions of cancer, lactose synthesis and treatment of chronic stress-induced disease	Zydney, 1998; Cowan & Ritchie, 2007; Madureira et al., 2007
Lactoferrin	- High iron-binding affinity - Regulation of iron absorption and immune responses - Exhibit antioxidant activity and has both anticarcinogenic and anti-inflammatory properties - Used in skin care cosmetics, special therapeutic diets for the relief of inflammation in dogs and cats - As supplemented infant formula	Tomita et al., 2002; Garcia-Montoya et al., 2012

Table 2.2 (continued)

Compositions	Functions	References
Immunoglobulins (IgG, IgA, IgM)	- Prevents mucosal infections by agglutinating microbes - High valency of antigen-binding sites - Prevent adhesion of microbes to surfaces, inhibiting bacterial metabolism by blocking enzymes, agglutinating bacteria and neutralizing toxins and viruses	Hurley & Theil, 2011; El-Loly, 2007
Bovine serum albumin	- Anti-mutagenic function and fatty acid binding - Ability to inhibit tumor growth	Madureira et al., 2007
Lactoperoxidase	- Catalyzed oxidation of thiocynate by hydrogen peroxidase and generates immediate products with antibacterial properties - Preserve raw milk quality during storage or transportation to processing plant	Zydney, 1998; Watanabe et al., 2000; Seiful et al., 2005

2.3 Introduction to Membrane Process

Membrane process is widely applied for the separation and purification in upstream and downstream processing. Microfiltration and ultrafiltration is the commonly used membrane process in industry. The performance of both type membranes is of high throughput however it is relatively low in terms of resolution and purification basis (Saxena et al., 2009). From years to years, membrane technology begins to evolve and become an emerging tool used mainly in food industry with 20 to 30% of the current €250 million turnover of membranes is used in the manufacturing industry globally (Daufin et al., 2001). The membrane process is now developing and growing rapidly from pressure gradient types to the existing electrical gradient principles as illustrated in Figure 2.3.

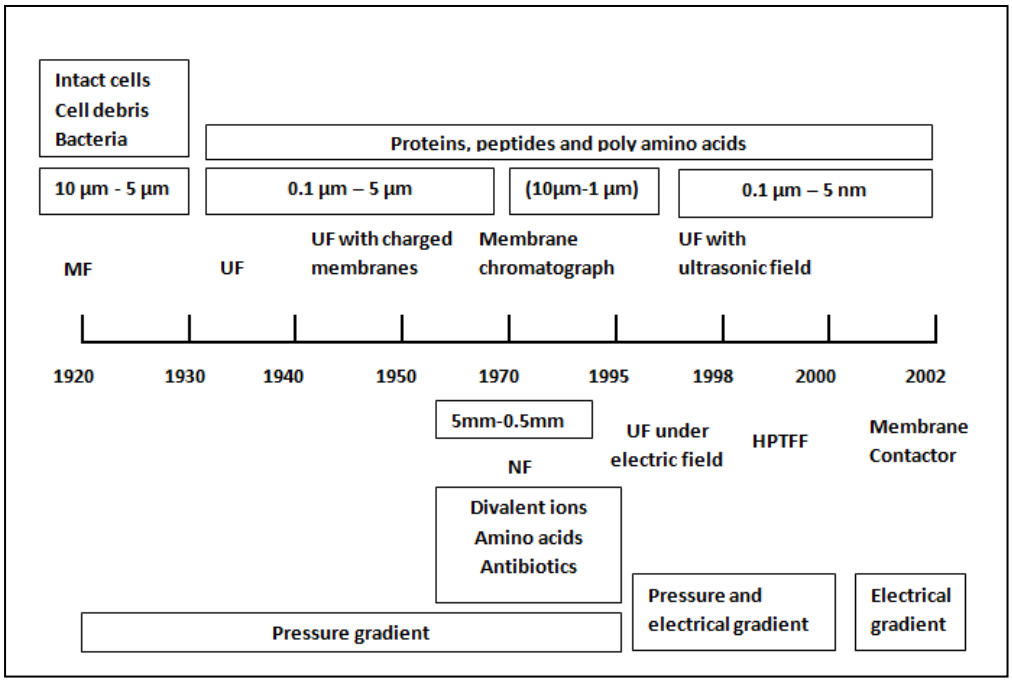

Figure 2.3 Milestone in development of membrane technologies for protein purification (Saxena et al., 2009)

The rapid development of membrane technology is due to its unique separation principle which is selective transport and efficient separation compare with other type of unit operations. A membrane actually acts as an interphase usually in the form of heterogeneous whereby it acts as a barrier to the flow of molecular and ionic species present in the liquid or vapors contacting the two surfaces (Saxena et al., 2009).

There are several advantages of using membrane as the separations process in industry. Membrane does not involve any phase changes or chemical additives, simple and easy to operate. Besides that, it allows for ease of scale up in industry production rate and able to decrease the equipment-size (Drioli, 2004). In addition, the separation process can be performed isothermally at relatively low temperatures with less energy consumption compared to other thermal separation process (Saxena et al., 2009).

2.3.1 Pressure-Driven Based Membrane Process

Membrane process can be classified according to different driving forces which are pressure gradient and electrical gradient. For pressure driven force, the pressure used depends on the pore size of the membrane and has to be adjusted as a function of the concentration rate desired (Langevin et al., 2012). Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) operate principally according to pressure driving force in various applications of both upstream and downstream processing. Figure 2.4 illustrates the membrane process classification according to size of solute to be separated.